PROTOCOL FOR SUBRENAL IMPLANT AND CHEMOTHERAPY OF THE HUMAN BRONCHIOLO-ALVEOLAR CARCINOMA H358

MODEL: (3JEG5) Subrenal Capsule Human Bronchiolo-Alveolar Carcinoma H358 Xenograft (delayed treatment)

Origin of Tumor Line: (No details.)

Summary of Test Procedures: A tumor fragment is implanted under the capsule of the kidney of either athymic Swiss (Cr:NIH(S)-nu) or athymic random bred (NCr-nu) mice. I.P. test agent treatment starts 4 days after tumor implant and is repeated every fourth day for a total of 4 injections. The parameter is change in tumor weight.

ANIMALS: (refer to Protocol 8)

Propagation and Testing: Athymic Swiss (Cr:NIH(S)-nu) or athymic random bred (NCr-nu) mice.

Weight: Mice should have a minimum weight of 18 gm for males and 17 gm for females.

Age: Record age of mice.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

EXPERIMENT SIZE:

General Testing: Six animals per test group and 10 animals per control group and 10 animals for an early control group. Typically, a single

test agent experiment is run with 4 dose levels of the test agent at 50% intervals. Total number of mice in a typical single test agent experiment (tests, control, and early control) is 44.

TUMOR TRANSFER: (refer to Protocols 2, 5, and 6)

PROPAGATION

Fragment: Prepare a 2x2x2 mm fragment of s.c. donor tumor.

Time: When donor tumor reaches 300-500 mg (approximately Day 28).

Site: Implant fragment s.c. into axillary region with puncture in inguinal region using a 13-gauge trocar.

TESTING

Fragment: Prepare a 19x19x19 Ocular Micrometer Unit (OMU) fragment. Average diameter must be 17-21 OMU's measured under a dissecting microscope. 10 OMU's = 1 mm.

Anesthetic: Any satisfactory anesthetic (e.g., chloral hydrate, Avertin, etc.).

Medium: Tissue culture medium with no antibiotics (e.g., 199, Eagles MEM, or Earles).

Time: When donor tumor reaches 300-500 mg (approximately Day 28).

Site: Implant fragment under the subrenal capsule, using a 16-gauge trocar with a 22° bevel, after exposing the kidney with a 7 mm dorsal skin incision. The wound is closed with a 9 mm wound clip after closing the peritoneum with 1-4 silk sutures.

TESTING SCHEDULE: (refer to Protocols 3 and 4)

Day 0: Anesthetize animals. Implant tumor and measure. Randomize animals after they recover from the anesthetic. Run bacterial cultures (refer to Protocol 7). Determine solubilities of test agent. Record deaths daily.

Day 1: Check cultures. Discard experiment if contaminated.

Day 2: Recheck cultures. Discontinue if contaminated and report accordingly.

Day 4: Record body weights (Weigh Day 1). Sacrifice early control group, measure tumors in OMU's. Calculate mean tumor weight. Record this mean tumor measurement as initial day (Day 4) tumor measurement for control group.

To provide estimated initial measurements for the treated groups, eliminate the measurements for the two largest and the two smallest tumors in the early control group. The mean tumor weight of the remaining six mice is designated the initial weight (Day 4) for all treated groups. Prepare test materials. Initiate i.p. test agent injections based on individual body weight. Treatment is q4d on Days 4, 8, 12 and 16. Prepare test agent fresh on each injection day and administer based on individual body weight for that day.

Days 8, 12 and 16: Prepare test agent fresh on each injection day and administer based on individual body weight for that day.

Day 19: End and evaluate experiment. Record body weights (Weigh Day 2). Measure tumors in OMU's and record. Final evaluation day

for this model is also test toxicity day, test no-take day, control early-death day, control no-take day, and Weigh Day 2.

QUALITY CONTROL:

(1) Positive control compound for this tumor system has not been established as of this publication date.

(2) Implant 2 or 3 additional mice which can be used for replacements in the event of surgical deaths. If surgical deaths do not occur, use these mice as additional control animals.

(3) Within a given experiment, whenever possible use mice from the same supplier, date of receipt, and shipping crate to reduce fighting. If mice fight, house fighters individually.

(4) House mice 3 to 6 per cage.

(5) Donor tumor should weight between 300-500 mg and be scrupulously cleaned of necrotic and/or hemorrhagic areas.

(6) In case of unusual deaths, these animals should be autopsied and peculiarities noted.

(7) Specific definitions for subrenal capsule implants for Control Status Code assignments by the computer (refer to Protocol 7.7) are:

(a) Acceptable control mean tumor weight change is equal to or greater than one mass doubling or 0.3 $\log_{10}$ increase between Day 4 and Final Evaluation Day.

(b) Control no-take: A mouse with a tumor weight increase of <20% between Day 4 and Final Evaluation Day. (Computer determined.)

(c) Excessive control no-takes: 4 or more no-takes are excessive in a control group of 10 mice (refer to Protocol 7.3).

(d) Excessive control early deaths: 2 or more control deaths in a group of 10 to 12 animals (i.e., >10%) on or before Final Evaluation Day that are not attributable to surgery or accident.

EVALUATION: (refer to Protocol 11)

The parameter measured is mean tumor weight change (delta) based on length and width measurements in millimeters. Compute mean animal body weights for Day 4 and Day 19, compute T/C for all test groups with 67% survivors on Day 19. An excessive body weight loss 5.0 g may also be used in evaluating toxicity.

The NCI screening laboratories are to measure and input OMU length and width measurements for tumors on Day 4 (measurements obtained from early control group, Day 4) and on Final Evaluation Day. The dimensions are measured and recorded in Ocular Micrometer Units (OMU). (They will be entered on the WS 180 Solid Tumor Data Form using type 2 with code H, per instructions of 9/81 from the Screener Instructions for Use of the Solid Tumor Input Form, section 3.3.1.2. By convention, the length (L) dimension must be entered first). The NCI computer:

(1) Converts OMU's to millimeters (mm).

(2) Calculates tumor weights (mgs) from tumor dimensions (mm x mm) following the formula for the volume of a prolate ellipsoid:

$\frac{L \times W^2}{2}$ Where L is the longer of the two measurements

(3) Calculates the change (delta) in mean tumor weight for each group of mice:

Change in Mean Tumor Weight =

Mean Tumor Weight$_{FINAL}$ - Mean Tumor Weight$_{INITIAL\ (Day\ 4)}$.

(4) Calculates the change (delta) in mean tumor weight for test (T) and control (C) groups.

(5) Calculates T/C% for all test groups with >67% survivors on Final Evaluation Day:

$$T/C\% = \frac{WtT}{WtC} \times 100 \text{ -- if WtT positive.}$$

$$T/C\% = \frac{WtT}{\text{Test Mean Tumor Weight}_{INITIAL\ (Day\ 4)}} \times 100 \text{ -- if WtT negative.}$$

CRITERIA FOR ACTIVITY:

An initial T/C of 1 to 20% is considered necessary to demonstrate moderate activity (MC-1). A reproducible T/C $\leq$0% is considered significant activity (DN-2).

REPORTING OF DATA: On the final day of testing, prepare final control and test reports. Input data. Screener assigns a code of "U" to an individual mouse

whose response screener considers invalid, including the following circumstances:

(1) Tumor lost from site of implant and kidney appears normal.

(2) Accidental injury or death.

(3) More than 1 tumor present.

(4) Infection at site of implant.

(5) Kidney does not appear normal.

A comment must accompany all "U" code designations. The screener designates as unsatisfactory (assigns a Test Status Code of "33") all test groups that the screener considers invalid for any reason, including a case where more than 33% of the mice have been assigned a "U" code. The computer designates as unsatisfactory (assigns a Test Status Code of "34") all tests where:

(1) There is no control delta calculated.

(2) More than 33% of test mice have been assigned a "U" code.

(3) Less than 67% of test mice are acceptable for calculation (i.e., initial tumor diameters are between 17 and 21 OMU's and final measurements or complete tumor regressions were observed on the Final Evaluation Day).

The computer assigns the appropriate Control Status Code to reflect the acceptability of the control group, using definitions listed under Protocol 7 (Tumor Quality Control) including the codes cited in section 7.7 and Instruction 14.

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